we determined that hydrogen gas, mainly originating from aliphatic C-H bonds, is a major cause of global radiation damage in protein crystals [1]. At cryogenic temperatures the gas remains inside the crystals causing lattice deformations by the generation of an inner pressure. This results in the well known decrease of diffraction power with increasing dose.

X-ray diffraction experiments, performed in order to directly identify the location of hydrogen abstraction, have been difficult. This is due to the small hydrogen X-ray scattering lengths. In an X-ray irradiation experiment on the polypeptide Cyclosporine A, we were able to indirectly observe hydrogen abstraction by X-ray induced bond lengths changes [2].

To further investigate this effect we have performed a combined synchrotron X-ray irradiation - neutron diffraction experiment. The results confirmed our findings from the previous X-ray diffractions experiments and further revealed that X-ray induced hydrogen abstraction is highly selective process.

[1] A. Meents, S. Gutmann, A. Wagner, C. Schulze-Briese. *PNAS* **2010,** *107,* 1094-1099. [2] A. Meents, B. Dittrich, S. Gutmann. *J. Synchrotron Rad.* **2009,** *16,* 183-190.

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Temperature and Time Dependent Studies of Radiation Damage

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Temperature-dependent X-ray crystallography has been used to characterize the time, space and energy dependence of radiation damage to protein crystals.

The sensitivity of global damage to protein crystals exhibits a dynamical transition near 200 K [1]. Below the transition, an activation energy for damage of ~1 kJ/mol, similar to that for solvent-free small molecule organic crystals, is observed, and may be associated with vibrationally assisted reactions. Above the transition, the activation energy of ~18 kJ/mol is similar to that for diffusive motions in the protein and solvent.

These diffusive motions continue after the X-rays have been shut off, and from 300 K to 180 K we observe "dark progression" of radiation damage. The rate of dark progression has an Arrhenius temperature dependence with an activation energy of 15 kJ/mol,and its timescale decreases from ~1000 s at 180 K to ~10 s at 300 K, suggesting the feasibility of outrunning radiation damage using faster data collecton. At intermediate temperatures (200-240 K), faster data collection does appreciably reduce radiation damage. But at room temperature, the timescales for the dominant diffusive damage processes are less than 2 s, and damage shows no dose rate dependence for dose rates between 8 and 300 kGy/s.

The spatial distribution of damage within the unit cell varies with temperature. At low temperatures, disulfide bridges and crystal contacts are readily damaged. But at 240 K and above, where diffusive motions are important and overall radiation sensitivity is much greater, solvent-exposed turns are the most sensitive while buried residues and residues involved in crystal contacts are more stable. These observations add detail to the common notion that damage at room temperature is due to diffusive motions.

Finally, 19 small molecule compounds, most known to be effective free-radical scavengers in solution, have been examined for possible protective effects in protein crystals. At room temperature, none significantly reduces radiation damage, and several increase it; at T=100 K, no protective or sensitizing effects are observed. Scavengers are ineffective in protecting protein crystals because a large fraction of the incident radiation is absorbed by protein atoms and because the ratio of scavenger molecules to protein molecules is too small to provide appreciable competitive protection.

This work was conducted at the MacCHESS facility at CHESS and in collaboration with IMCA-CAT at the APS.

[1] M. Warkentin, R.E. Thorne, *Acta Crystallographica Section D-Biological Crystallography* **2010,** *66*, 1092-1100.

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MS

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Combining X-Ray Diffraction and *in-situ* Spectroscopy to Study Haem Proteins

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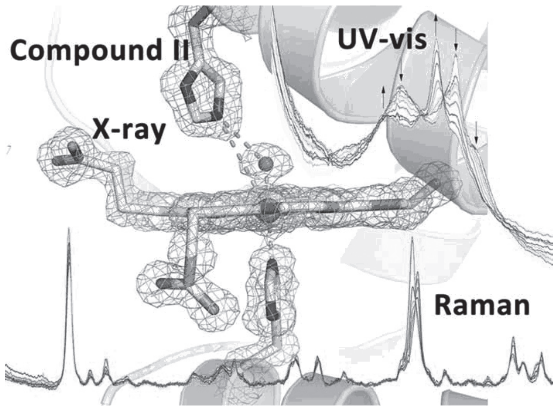
The influence of X-ray radiation damage to protein crystals is well known to occur even at cryogenic temperatures, and redox active sites like metal sites seem especially vulnerable for radiation-induced reduction. It is essential to correctly know the oxidation state of these metal sites in protein crystal structures, to be able to interpret the structure-function relation.

We have used *in-situ* (online) UV-vis and Raman spectroscopy to study how different oxidation states of the haem proteins myoglobin and catalase-peroxidase are influenced by X-rays during crystallographic data collection. The spectroscopic changes have been monitored as a function of X-ray exposure (dose absorbed), and show that the different redox-states in myoglobin vary in how fast they are "reduced" by the X-rays (e.g. ferric Fe^{3+} myoglobin is reduced faster than ferryl Fe^{IV}=O myoglobin) [1], and there is also differences between ferric myoglobin and catalase-peroxidase. The higher oxidation states of myoglobin are not reduced to normal ferrous Fe^{2+} or ferric Fe^{3+} states, but end up in some intermediate state. One of the primary goals of the project has been to characterise and study the different intermediates in the reaction between myoglobin and peroxides [2], [3]. The reaction intermediates generated in this reaction appear biologically relevant since myoglobin is proposed to function as a scavenger of reactive oxygen species during oxidative stress.



We have also been able to use the radiation damage to generate an